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Atty. Docket No.: 9409/2092 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Communi et al	Examiner:	Li, Ruixiang
Serial No.:	09/924,125		
Filed:	August 7, 2001	Group Art Unit:	1646
Entitled:	The Natural Ligand for Orphan G-protein Coupled Receptor GPR86 and Methods of Use		
		Conf. No.:	3058

Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450

RULE 132 DECLARATION OF DR. JEAN-MARIE BOEYNAEMS

I, Jean-Marie Boeynaems, hereby declare that:

1. I am an inventor of the above referenced patent application. I received a M.D. from the Free University of Brussels (ULB) in 1975 and a Ph.D. from the Free University of Brussels (ULB) in 1981. I am currently a Professor of Pharmacology at the Free University of Brussels (ULB), head of the Laboratory of Medical Chemistry at the Erasme Academic Hospital in Brussels, and am a member of the Belgian Royal Academy of Medicine. To date, I have authored over one hundred publications (approximately 150) in international scientific journals.

2. I have read the Office Action mailed in the above-referenced patent application on August 21, 2003, and understand that questions have been raised by the Examiner as to whether the newly de-orphanized receptor GPR86 has either a well established utility, or a specific, substantial and credible utility. I understand that it is the Examiner's position that we may establish a patentable utility for the GPR86 receptor by showing some physiological function of the receptor.

3. The experiments described herein (results shown in Exhibit A) were performed under my direction. As taught in the present patent application (Example 2), the GPR86 receptor is expressed in immune cells, with high levels of expression identified in the spleen. Consistent with these teachings, recent studies have shown that the GPR86 receptor is expressed preferentially in dendritic cells (Zhang et al., 2002, *J. Pharmacol. Exp. Ther.* 301: 705-713). The

experiments described below demonstrate that ADP induces the phosphorylation of ERK1 in human dendritic cells, an event which has been shown to inhibit IL-12 production. The ADP-induced phosphorylation of ERK1 was blocked by the GPR86 receptor antagonist AR-C69931MX, implicating the GPR86 receptor as the origin of the ADP-induced ERK1 phosphorylation event. In mouse dendritic cells, we observed that AR-C69931MX potentiates the production of IL-12 in response to CD40 ligand stimulation, indicating that GPR86 plays an inhibitory role in IL-12 cytokine production. This is consistent with the teaching in the specification at page 43, lines 17-21 that "GPR86, which is expressed in cells of the lymphocyte lineages...spleen as well as leukemic cells, can have a role in immune processes".

Exhibit A shows the results of experiments conducted following the protocols described hereinbelow, in which ADP was shown to phosphorylate ERK1, and in which the GPR86 receptor antagonist AR-C69931MX was shown to potentiate the production of IL-12 in response to CD40 ligand. The experiments described herein were performed as described in detail in the above-referenced patent application.

GPR86-mediated ERK1 phosphorylation

The present application teaches that the GPR86 receptor is expressed in immune cells, consistent with a role for GPR86 in modulation of mammalian immune responses. More recent data has identified GPR86 expression in dendritic cells (Zhang et al., 2002, *J. Pharmacol. Exp. Ther.* 301:705-713). Accordingly, the following studies were performed to confirm a role for GPR86 in the cellular physiology of immune cells.

As previously described (Puig-Kroger et al., 2001, *Blood* 98:2175) ERK1/2 signaling plays a key role in the regulation of cytokine production by dendritic cells following their activation with LPS or TNF-a. Moreover our group previously showed that ADP can induce the phosphorylation of ERK1/2 through the stimulation of human GPR86 (Communi et al., 2001 *J. Biol Chem.* 276:41479). Considering these data we stimulated immature dendritic cells with ADP, ADPbS and 2MeSADP for different times and concentrations. As

shown in Exhibit A, figure 1B, the phosphorylation was already detectable at 1 mM for all the compounds. The GPR86 receptor antagonist AR-C69931MX (10 μM) totally inhibited ERK1 phosphorylation, whereas the $P2Y_1$ receptor antagonist MRS-2179 (10 μM) had no effect (Exhibit A, figure 1C). AR-C69931MX also inhibited ERK1 phosphorylation at 1 μM (data not shown).

GPR86-regulated modulation of IL-12

Given the role of ERK1/2 phosphorylation in the regulation of cytokine production by dendritic cells following LPS or TNF-α activation, and the high levels of expression of GPR86 on dendritic cells, we endeavored to examine the effect of ADP signaling through the GPR86 receptor on cytokine physiology in mouse dendritic cells.

CD11c+ leukocytes were isolated from C57BL\6 spleen by incubation with magnetic microbeads conjugated to a monoclonal hamster anti-mouse CD11c antibody (Mitenyi Biotech). Freshly isolated cells were cocultured in complete RPMI 1640 medium with irradiated 3T3 (left part) or with irradiated 3T3-CD40L (right part) for 24h. Cell-free supernatants were collected and IL-12p70 concentration was determined by ELISA (eBioscience). The data shown in Figure 2 of Exhibit A demonstrate that in mouse dendritic cells, the GPR86 antagonist AR-C69931MX potentiates the production of IL-12 in response to CD40 ligand stimulation, indicating that endogenous ADP, acting via the GPR86 receptor, exerts an inhibitory effect on that production (βS = ADPβS). Therefore, a GPR86 antagonist may be valuable as a vaccine adjuvant, by amplifying the production of IL-12 and promoting the Th1 polarization of $CD4^+$ T lymphocytes.

Taken together, these data implicate GPR86 as mediating physiological levels of cytokine production in immune cells, and suggest that GPR86 may be a valuable target for the development of compounds which can be utilized to regulate the immune response in mammals.

February 18, 2004
Date

Jean-Marie Boeynaems

EXHIBIT A

Figure 1

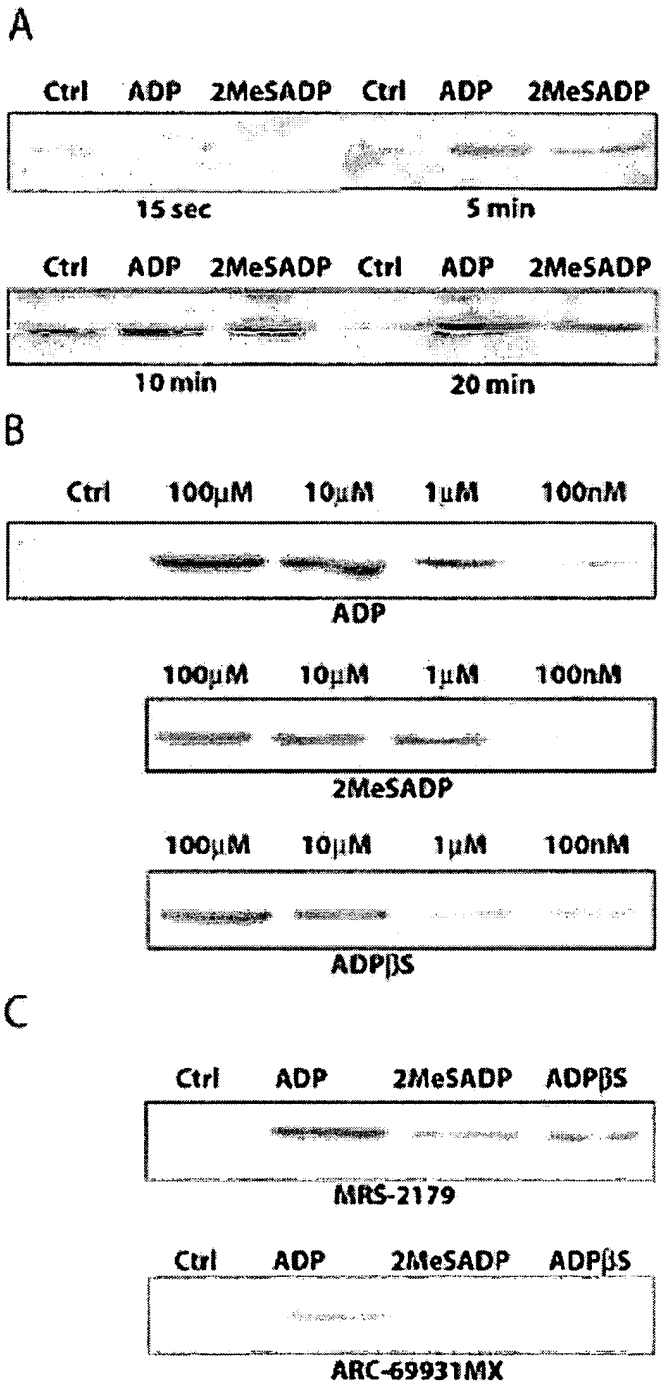

Figure 2